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A MUTANT OF *NANNOCHLOROPSIS* DEFICIENT IN EICOSAPENTAENOIC ACID PRODUCTION

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Key Word Index—*Nannochloropsis*; Eustigmatophyceae; biosynthesis; lipids; fatty acids; arachidonic acid; eicosapentaenoic acid; mutant; gamma ray mutagenesis; omega-3 desaturase.

Abstract—The most abundant fatty acids found in *Nannochloropsis* are 16:0, 16:1 (*n*-7) and 20:5 (*n*-3). By analysing the fatty acid composition of mutagenized cells by gas chromatography, we discovered a mutant strain of *Nannochloropsis* that is completely devoid of 5,8,11,14,17-*cis*-eicosapentaenoic acid [20:5 (*n*-3)]. An analysis of total fatty acids from whole cells showed that the loss of 20:5 (*n*-3) in mutant cells was accompanied by a concomitant increase in the relative amount of 20:4 (*n*-6). However, the increase in 20:4 (*n*-6) was not proportional to the loss of 20:5 (*n*-3) within individual lipid classes. The percentage of 20:4 (*n*-6) in monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol and phosphatidylglycerol (PG) in the mutant strain was lower than the wild type levels of 20:5 (*n*-3) in these lipids. Conversely, the percentage of 20:4 (*n*-6) in phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in the mutant cells was higher than the wild type levels of 20:5 (*n*-3). Molecular species analysis of MGDG showed that, compared to the wild type strain, the mutant strain contained more 16:1/16:0 MGDG, and less of the MGDG species containing C_{20} fatty acids. Relatively low levels of 20:4/20:4 MGDG were found in the mutant, although 20:5/20:5 MGDG was abundant in wild type cells. Relative membrane lipid levels were altered in the mutant compared to the wild type strain, particularly PG, which showed a fourfold decrease. The mutant cells grow more slowly than the wild type cells on both solid and in liquid media. Electron micrographs showed that the mutant strain had fewer thylakoid stacks per chloroplast, and fewer thylakoids per stack than wild type cells. We concluded that the mutation affects an extrachloroplastic (*n*-3) desaturase that is required for the synthesis of 20:5 (*n*-3), which is found in both chloroplast and extrachloroplast lipids.

INTRODUCTION

The membrane lipids of *Nannochloropsis* sp. (Eustigmatophyceae), an oleaginous, eukaryotic marine alga, contain very-long-chain polyunsaturated fatty acids (VLC-PUFAs). Eicosapentaenoic acid [or 20:5 (*n*-3)] is the major VLC-PUFA produced by *Nannochloropsis*; it has value both for its nutritional component when used as aquaculture feed and for its potential pharmaceutical use in humans [1].

A detailed model has been developed for C_{18} polyunsaturated fatty acid synthesis in higher plants, but the features of 20:5 (*n*-3) synthesis in eukaryotic algae is largely unknown. Previous work has shown that 20:5 (*n*-3) is synthesized from both 20:4 (*n*-6) and 20:4 (*n*-3) in the algae *Phaeodactylum tricornutum* [2] and *Porphyridium cruentum* [3]. In addition, fungi such as *Mortierella alpina* [4] and *Saprolegnia parasitica* [5] use 20:4 (*n*-6) as a substrate for synthesis of 20:5 (*n*-3). Schneider and Roessler [6] proposed that 20:5 (*n*-3) was synthesized from 20:4 (*n*-6) based on radiolabelling studies and analysis of the fatty acid composition of membrane lipids. Although the glycerolipid substrates for this desaturation reaction in *Nannochloropsis* are not known with certainty, these studies suggested that phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were intermediates in the formation of VLC-PUFAs.

The analysis of mutant strains has been beneficial for developing models for lipid biosynthetic pathways and cloning desaturase genes from higher plants [7], cyanobacteria [8] and fungi [9]. This paper reports the isolation and characterization of a mutant strain of *Nannochloropsis* that is completely devoid of 20:5 (*n*-3). The alteration in fatty acid composition of the mutant membrane lipids indicated that 20:5 (*n*-3) is synthesized only in an extrachloroplastic location.

RESULTS AND DISCUSSION

Nannochloropsis was mutagenized by exposure to gamma irradiation or ethyl methane sulphonate (EMS). The proportions of cells that could form colonies after being exposed to the mutagens for different amounts of time decreased linearly and levelled off at about 1% at the highest treatment levels. To increase the chance of

finding a 20:5 (*n*-3)-deficient mutant strain, colonies were screened from treatments that resulted in a low survival percentage (14% survival for the gamma ray treatment and 12% survival for the EMS treatment) and thus a high mutation rate. The fatty acid composition of 1090 single colonies, approximately half from each mutagenic treatment, was examined by GC.

Of the 20 colonies with apparent alterations in the proportion of fatty acids in the initial screen, only one maintained the alteration upon rescreening. This colony (strain JS1, from the gamma ray treatment) had no detectable 20:5 (*n*-3) and an increased proportion of 20:4 (*n*-6) compared to the wild type (Fig. 1). This change in the level of fatty acids in the mutant supports our model that the substrate for the formation of 20:5 (*n*-3) is 20:4 (*n*-6) [6]. In addition, pulse-chase labelling studies support the idea that 20:4 (*n*-6) is an intermediate, and not an end product, of VLC-PUFA synthesis; in wild type cells, the amount of label incorporated from [^{14}C]acetate into 20:4 (*n*-6) decreased during a chase of several hours, whereas the amount incorporated into 20:5 (*n*-3) increased (data not shown). The increased amount of 20:4 (*n*-6) in the mutant strain suggests that JS1 is defective in a desaturase that incorporates a double bond at the (*n*-3) position of 20:4 (*n*-6) to form 20:5 (*n*-3). Of course, the same phenotype could result from a mutation in, for example, a regulatory or cofactor gene which is uniquely required for 20:5 (*n*-3) synthesis; however, this seems less likely, because the synthesis of other VLC-PUFAs presumably would require the same gene products.

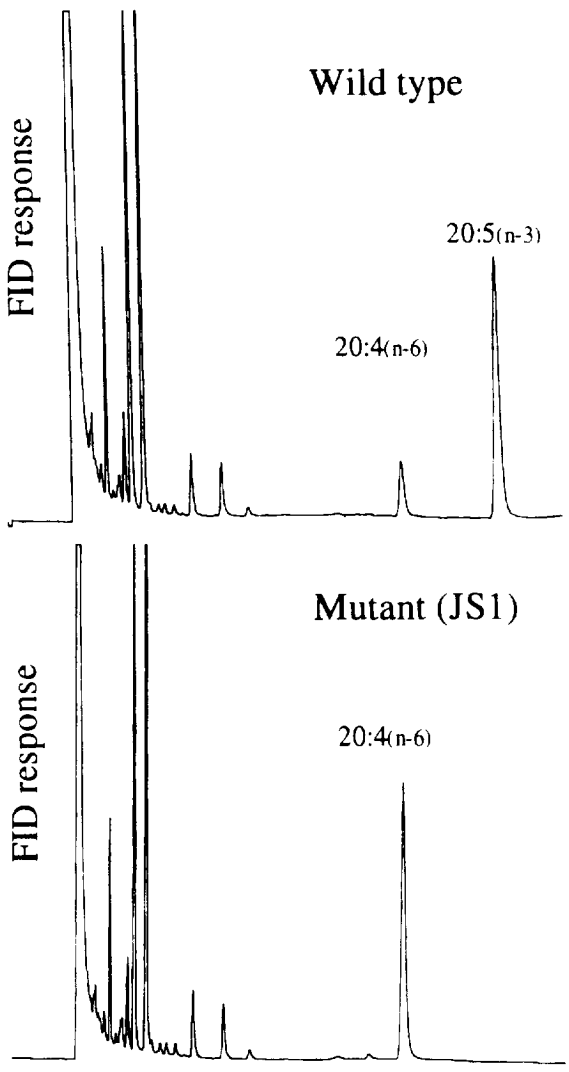

Fig. 1. GC of fatty acids from wild type and mutant (JS1) cells. Lipids from whole cells were transesterified to produce FAMEs and analysed by GC as described in the Experimental section. Chromatograms from the wild type (top) and 20:5 (*n*-3)-deficient mutant JS1 (bottom).

The total fatty acid composition of the mutant strain is very different from that of the wild type strain. The fatty acids 14:0, 16:0 and 16:1 (*n*-7) are the next most abundant fatty acids after 20:5 (*n*-3) in *Nannochloropsis* [10]. The mutant strain has a higher proportion of these fatty acids, in part because of a large increase in triacylglycerol (TAG) relative to the membrane lipids; TAG from the mutant strain contains 24.9% of the total fatty acids found in glycerolipids compared to 7.2% in the wild type strain. TAG from mutant cells contains more 14:0 and less 16:0 and 16:1 (*n*-7) than wild type cells (Table 1). The proportion of these three fatty acids in the membrane lipids is altered in the mutant strain. All lipids except PE have a higher proportion of 14:0 in the mutant compared to the wild type strain. Monogalactosyldiacylglycerol (MGDG) from the mutant strain contains more 16:0, whereas phosphatidylglycerol (PG), PC, PE and phosphatidylinositol (PI) contain less. The chloroplast lipids MGDG, digalactosyldiacylglycerol (DGDG) and PG from the mutant strain contain more 16:1 (*n*-7), whereas PC, PE, diacylglyceroltrimethylhomoserine (DGTS), PI and sulphoquinovosyldiacylglycerol (SQDG) contain less.

The C_{18} fatty acids do not normally accumulate to a large extent, except in PC; they were previously proposed to be intermediates in the pathway of 20:5 (*n*-3) synthesis [6]. However, the mutation has variable effects on the accumulation of C_{18} fatty acids in different lipids and their proportions do not uniformly increase in JS1. In general, the lipids from whole cells of the mutant strain contained higher proportions of 18:1 (*n*-9), and accumulation of this fatty acid increased in most lipids. The fatty acids 18:2 (*n*-6) and 18:3 (*n*-6) did not change significantly overall, but were found in lower proportions in PC, PE and PI and in higher proportions in PG, MGDG and DGDG from the mutant strain. The increase of these two fatty acids in chloroplast lipids and decrease in extrachloroplast lipids may indicate that they are preferentially used for transport into the chloroplast.

The largest changes occurred in the proportion of 20:4 (*n*-6) and 20:5 (*n*-3). Although 20:5 (*n*-3) is a major fatty acid in the wild type strain, no trace of it was found in the mutant strain. The percentage of 20:4 (*n*-6) increased in all lipids in the mutant, but not always in proportion to the loss of 20:5 (*n*-3). The fatty acid 20:3 (*n*-6) also increased in every lipid in which it was measured. Com-

Table 1. Fatty acid composition of lipids and proportion of membrane lipids from wild type (WT) and mutant (JS1) strains of *Nannochloropsis*

		Percentage membrane lipid (SD)†	Fatty acid composition of lipids (mol %)*									
			14:0	16:0	16:1 (*n*-7)	18:0	18:1 (*n*-9)	18:2 (*n*-6)	18:3 (*n*-6)	20:3 (*n*-6)	20:4 (*n*-6)	20:5 (*n*-3)
Whole cells	JS1		12.8	20.0	32.1	0.7	5.9	3.6	tr‡	1.4	23.3	
	WT		6.9	19.9	27.4	tr	1.7	3.5	0.7	tr	4.2	34.9
TAG	JS1		18.5	29.5	40.3	1.4	6.0	0.9	tr	1.3	2.0	
	WT		10.8	39.6	45.0	1.7	1.6	0.8				0.5
MGDG	JS1	31.4 (4.6)	21.5	18.8	37.2	tr	2.2	1.9	tr	0.7	17.3	
	WT	28.0 (0.5)	20.0	11.8	13.0	tr	tr	0.6			0.7	53.2
DGDG	JS1	6.3 (0.1)	10.3	28.9	50.0	0.6	2.6	2.2		tr	5.1	
	WT	14.0 (0.9)	8.0	30.7	40.7	tr	tr	1.0			tr	18.5
SQDG	JS1	11.0 (3.9)	12.9	49.1	36.9	1.0	1.0	tr			0.7	
	WT	8.1 (0.5)	5.5	52.0	41.3	0.6	tr					tr
PG	JS1	2.6 (0.1)	7.5	42.1	34.2	2.1	3.4	5.5			5.2	
	WT	10.0 (0.4)	1.7	51.8	14.3	0.7	tr	0.6				30.5
PC	JS1	26.0 (0.3)	3.5	10.8	27.2	tr	13.7	11.4	1.0	2.6	29.3	
	WT	17.4 (0.6)	2.6	14.1	46.7	0.6	6.6	17.4	3.4	tr	2.1	6.1
PE	JS1	8.1 (1.5)	2.1	3.4	9.8	tr	1.2	0.9		4.5	77.7	
	WT	6.4 (0.1)	2.7	8.6	28.3	0.7	1.7	2.4	1.0	2.2	25.9	26.5
DGTS	JS1	6.5 (0.3)	12.6	7.5	15.6	0.7	2.0	1.6		1.3	58.7	
	WT	10.4 (0.8)	3.7	6.9	23.5	0.8	0.9	1.3			5.0	57.8
PI	JS1	8.2 (1.8)	10.9	21.1	26.3	tr	5.3	2.5		tr	33.2	
	WT	5.7 (0.5)	4.4	28.6	36.1	1.1	3.9	4.9			14.1	6.8

* Average of two determinations. SDs were less than 10% of the given value for values greater than 5%.
† The total fatty acids in each lipid as a percentage of the total fatty acids in all membrane lipids.
‡ Trace (less than 0.5%).

pared to the wild type strain, the level of total C_{20} fatty acids in the mutant strain increased in TAG, PC, PE and PI, but decreased in MGDG, DGDG and PG (Fig. 2). Those lipids in which the C_{20} fatty acids increased are present in extrachloroplast membranes or, in the case of TAG, in cytosolic oil bodies. In contrast, those lipids in which they decreased are chloroplastic (although PG is also found in extrachloroplast membranes). Assuming that JS1 contains a mutation in a desaturase gene, the complete loss of 20:5 (*n*-3) implies that a single enzyme carries out the *n*-3 desaturation step in *Nannochloropsis*, unlike in higher plant cells, where the desaturation steps that produce PUFAs are carried out by duplicate pathways of microsomal and chloroplast enzymes. Plants with a single mutation that affects a desaturase in only one of these pathways still synthesize PUFAs, although in reduced amounts [11, 12]. In the other two species of algae for which data are available, 20:5 (*n*-3) is synthesized from both 20:4 (*n*-6) and 20:4 (*n*-3) [2, 3]. Analysis of the lipids in the mutant strain of *Nannochloropsis* supports the idea that there is only one pathway for synthesis of 20:5 (*n*-3). The substrate 20:4 (*n*-6) is present in every glycerolipid in the JS1 mutant strain, yet no 20:5 (*n*-3) is present. Two genes encoding different (*n*-3) desaturases are unlikely to be both mutated; therefore, the loss of 20:5 (*n*-3) implies a single locus for the gene encoding the (*n*-3) desaturase. The increased accumulation of C_{20} fatty acids in extrachloroplast lipids of the mutant suggests that this (*n*-3) desaturation reaction normally occurs on extrachloroplastic lipids, and then 20:5 (*n*-3), or lipids that contain it, is imported into the chloroplast for use in chloroplast lipid synthesis. Recent evidence from radiolabelling studies in the marine diatom, *P. tricornutum*, also suggested that desaturases that form PUFAs are not present in the chloroplast [13]. In that case, the C_{18} fatty acids and 20:4 (*n*-6) that were incorporated into the chloroplast lipids MGDG and PG were not desaturated; thus, 20:5 (*n*-3) attached to MGDG was proposed to originate in an extrachloroplastic location.

The relative proportions of lipid classes were altered in the mutant. As mentioned above, TAG contained 24.9% of the total fatty acids in the mutant strain, but only 7.2% of fatty acids in the wild type strain in cells harvested during exponential growth. The large amount of TAG in the mutant strain may be a result of the slower growth rate (see below), because wild type cells also have greatly increased TAG levels when cell growth is slowed by nutrient deficiency [14]. In comparing membrane glycerolipids (excluding TAG), the chloroplast lipids MGDG and SQDG were found in similar proportions in both strains: however, in the mutant strain, DGDG was reduced by half and PG was reduced fourfold (Table 1). Among extrachloroplast lipids, PC increased by 50% in the mutant strain, whereas PI increased and DGTS decreased slightly. Chloroplast lipids from wild type cells contain large amounts of 20:5 (*n*-3) and only trace

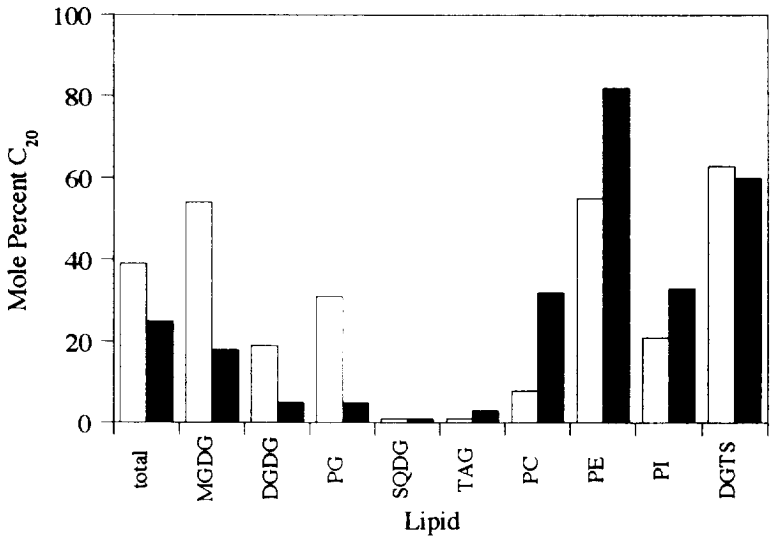

Fig. 2. The molar percentage of C_{20} fatty acids in total lipid extracts and individual lipids from wild type (white bars) or the 20:5 (*n*-3)-deficient mutant (black bars) cells. Lipids were extracted and separated and fatty acids were analysed as described in the Experimental section.

amounts of 20:4 (*n*-6). The decrease in the relative accumulation of chloroplast lipids in the mutant suggests that the synthesis of chloroplast lipids may be slowed by the abnormal glycerolipid intermediates containing 20:4 (*n*-6). The defect in growth rate in the mutant strain (see below) may be more a result of these large changes in relative lipid composition than the replacement of 20:5 (*n*-3) by 20:4 (*n*-6).

Pulse-chase radiolabelling of cultures with [^{14}C]acetate showed that *de novo* synthesis of lipids was altered in the mutant strain (Fig. 3). Previous radiolabelling studies on wild type cells showed that large changes in the amount of label occurred only in TAG, MGDG, PC and PE [6]; thus, labelling of these lipids was examined in JS1. Compared to wild type cells, more radioactivity accumulated in TAG from mutant cells at every point; this correlates with the accumulation of proportionally more TAG in the these cells. In the wild type strain, radioactivity in MGDG increased during the first 5 hr of the chase, whereas in the mutant strain radioactivity in MGDG increased only slightly early on and did not increase further. In both strains, PC was one of the most highly labelled lipids at the earliest point after cells were transferred into unlabelled medium (0.5 hr), and subsequently lost label during the 21-hr chase. In the mutant strain, the peak of radioactivity in PC was lower than in wild type cells and was obtained later during the chase. The decrease in labelling of PC and PE in the wild type cells partly corresponded with an increase in labelling of MGDG, but this was not true for the mutant cells. Because the increase in MGDG label is not sufficient to account for the decrease of label in PC, especially in the mutant cells, the destination of all fatty acids after their departure from PC is unclear. However, during the chase, there is an increase of label in an unknown lipid that migrates similarly to sterol esters (data not shown). The possibility that this lipid incorporates unsaturated C_{18} fatty acids from PC is being investigated.

In the mutant, the proportion of molecular species of MGDG containing C_{20} fatty acids declined in the mutant by 50% compared to the wild type. The major molecular species of MGDG containing 20:4 (*n*-6) were 20:4/16:1 (designating 20:4 in the *sn*-1 and 16:1 in the *sn*-2 position of MGDG) and 20:4/14:0 (Fig. 4), whereas in the wild type, the major species containing 20:5 (*n*-3) were 20:5/16:1, 20:5/14:0, and 20:5/20:5. Interestingly, only a low level 20:4/20:4 MGDG was detected in the mutant strain, even though 20:5/20:5 is an abundant species in the wild type strain. Its low abundance may indicate that the enzymes involved in forming galactolipids with two C_{20} fatty acids are specific for 20:5 (*n*-3) or that a requisite 20:4/20:4 lipid substrate was not available. The most abundant species in the mutant strain was 16:1/16:0 MGDG, making up about 60% of the total MGDG as compared to 24% in the wild type strain. The C_{16} fatty acids do not strongly incorporate [^{14}C]acetate even in wild type cultures [6]; the increased proportion of 16:1/16:0 MGDG in the mutant strain may explain why MGDG does not accumulate label to the same extent as in wild type cells (Fig. 3).

The increased proportion of 16:1/16:0 MGDG in the mutant indicates a change in the flux of fatty acids through the MGDG biosynthetic pathways. Unlike in higher plants, the presence of C_{16} fatty acids in extrachloroplast lipids makes it difficult to determine the source of lipids from their fatty acid composition alone. For instance, TAG is synthesized by the 'eukaryotic' pathway, yet the *sn*-2 position of TAG contains 16:1 exclusively [15]. It is possible, although not certain, that 16:1/16:0 MGDG is a 'prokaryotic' lipid synthesized by the chloroplast pathway. We speculate that the mutant contains an increased proportion of 'prokaryotic'

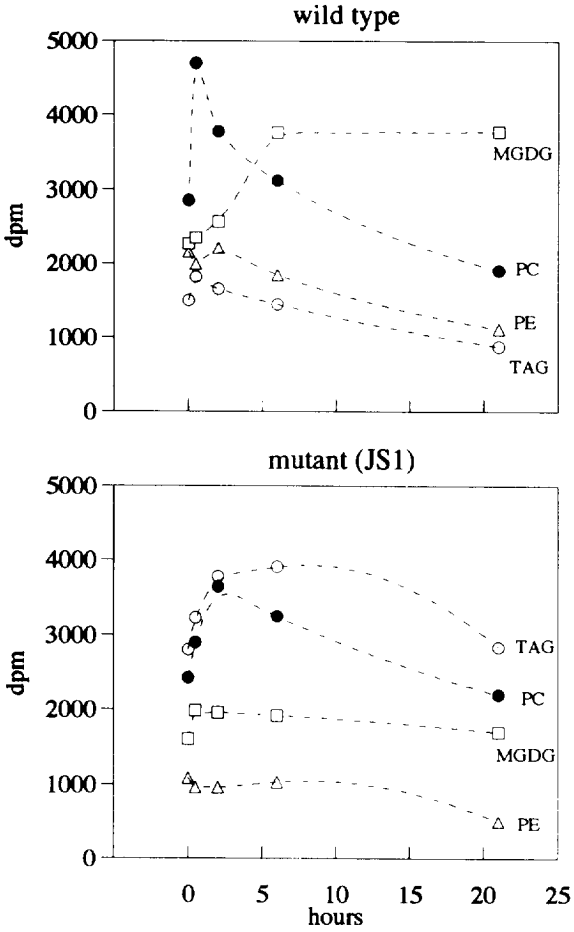

Fig. 3. Labelling of lipids from wild type and the 20:5 (*n*-3)-deficient mutant strain (JS1). Cultures were labelled with [^{14}C]acetate for 1 hr then were transferred to medium without label. Portions of the culture were harvested at various times, lipids were extracted and equal numbers of counts for each time point were separated by TLC as described in the Experimental section. The experiment was repeated twice. Results shown are from a representative experiment.

16:1/16:0 MGDG to compensate for decreased flux of VLC-PUFAs from the cytoplasm, which contribute to the bulk of MGDG in the wild type strain. VLC-PUFAs may be incorporated into MGDG by importing diacylglycerol (DAG) moieties from the cytoplasm as suggested for higher plants [16] or by a 'retailoring' process designed to exchange new fatty acids into existing MGDG molecules as suggested for *P. tricornutum* [13]. Retailoring is believed to occur in PC in higher plants as a mechanism to form PUFAs, and in *Dunaliella salina* [17] as a way to alter membrane fluidity in response to temperature changes.

Colonies of the JS1 strain growth on solid agar medium are smaller and paler than wild type colonies. No reversion of the growth or fatty acid phenotype was observed even after several passages of EMS-mutagenized or untreated JS1 cultures. Cultures of the mutant strain inoculated at low cell densities exhibited a longer lag phase than wild type cultures, and grew more slowly during exponential growth (Fig. 5). Although the slow growth may result from a lesion separate from the desaturation defect, mutations that change the proportion of particular PUFAs in membrane lipids alter growth rates at lower than normal growth temperatures in *M. alpina* [18], *Arabidopsis* [19, 20], tobacco [21] and *Anacystis nidulans* [22]. Alternatively, the lower growth rate could be ascribed to the altered relative accumulation in the mutant strain of the membrane lipids DGDG, DGTS, PC and especially PG, which showed a fourfold decrease (Table 1). The functions of algal membrane lipids other than their contribution to bilayer structure are currently unclear; however, in higher plants, PG is believed to be a component of the light harvesting complex II [23]. In addition, the proportion of MGDG in the mutant strain that contains PUFA decreased and was replaced by 16:1/16:0 MGDG (Fig. 4). This large alteration in the saturation level of fatty acids comprising MGDG and probably other chloroplast lipids may be expected to affect the efficiency of membrane processes.

To determine whether the reduced growth rate resulted from lower photosynthetic efficiency, the rate of oxygen evolution as a function of light intensity was examined. Although the rate of oxygen evolution by the mutant strain was always less per cell than that of the wild type strain at each light intensity, the maximum oxygen evolution of both cultures was obtained at the same light intensity (*ca* 200 μmol photons $m^{-2} min^{-1}$) and no photo-inhibition was found in either culture at light levels up to 700 μmol photons $m^{-2} min^{-1}$ (data not shown).

Typical electron micrographs of cells from mid-log cultures are shown in Fig. 6. The most obvious difference is the number of thylakoid stacks per chloroplast; the wild type strain averages eight stacks per chloroplast, whereas the mutant has only six stacks per chloroplast. In addition, the mutant strain contains an average of only two thylakoids per stack compared to three in the wild type cells. Presumably, the differences in thylakoid structure result from the different molecular species or altered proportions of chloroplast lipids in the mutant. Previous studies of wild type cells found that increased light intensity decreased these parameters; this decrease and other changes in the chloroplast membranes correlated with a decrease in the amount of galactolipids and increase in the amount of TAG per cell in high light [10]. Although the wild type and mutant cells were grown in identical low-light conditions, the chloroplast structure and the increased proportion of TAG found in the mutant cells are indicative of cells growing in high-light conditions; thus, the inability to fix carbon may not be the primary cause of the slow growth rate.

EXPERIMENTAL

Mutagenesis and screening. A culture of *Nannochloropsis* sp. was grown in artificial sea-water at 25° as described previously [6] until early log-phase (2.1×10^7 cells ml^{-1}). The cultures were mutagenized by

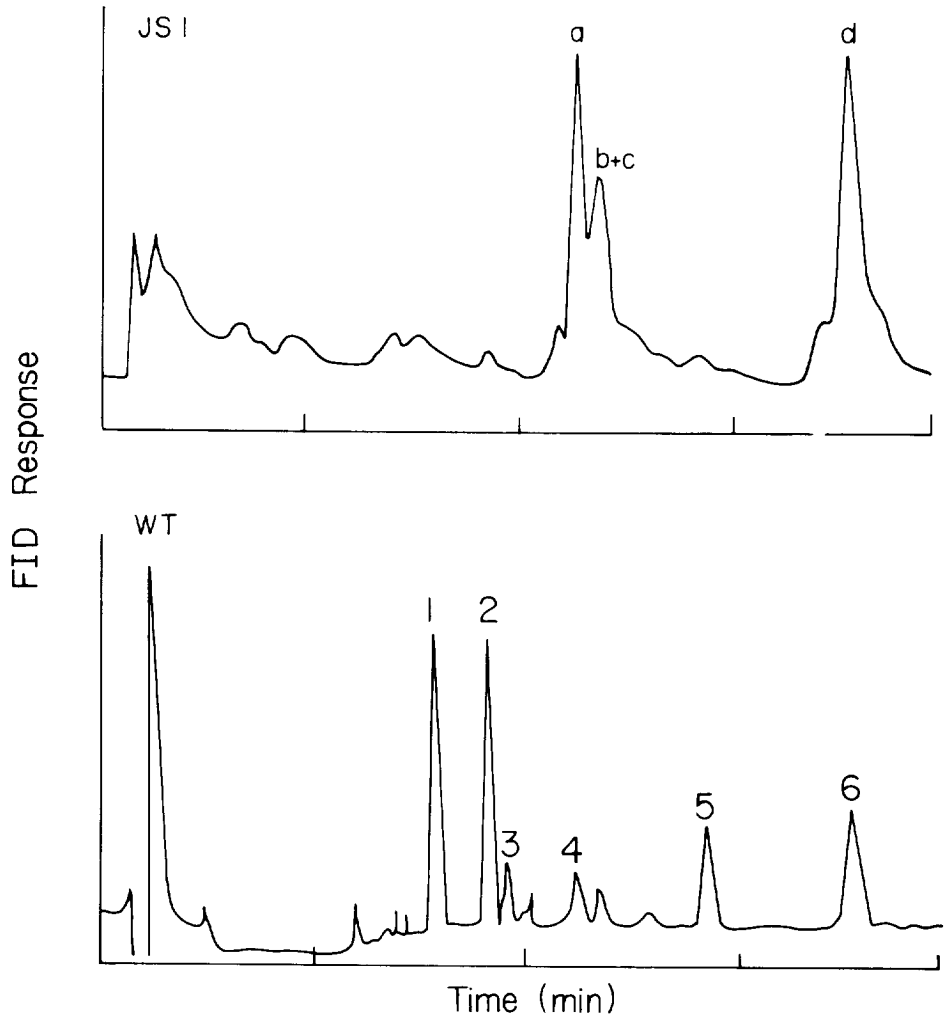

Fig. 4. Separation of MGDG molecular species by HPLC. Upper panel—mutant strain (JS1); lower panel—wild type strain (WT). Peak identification: (a) 20:4/16:1; (b) 20:4/14:0; (c) 20:4/20:4; (d) 16:1/16:0; (1) 20:5/20:5; (2) 20:5/14:0; (3) 20:5/16:1; (4) undetermined; (5) 20:5/16:0; (6) 16:1/16:0. Note that 20:4/20:4 MGDG is poorly separated from the 20:4/14:0 peak when FID is used. Detection by UV at 205 nm showed a relatively well separated peak which was confirmed as 20:4/20:4 by GC analysis.

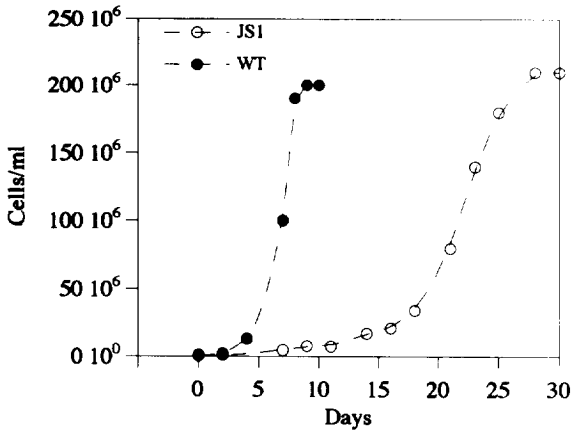

Fig. 5. Growth rates of wild type and the 20:5 (*n*-3)-deficient mutant strain. Cells were inoculated into artificial sea-water medium and grown with 1% CO_2 in air as described in the Experimental section: ●, wild type; ○, JS1 mutant.

exposure to gamma rays from a ^{137}Cs source (498 rad min^{-1}) or by incubation in 2.5% EMS with vigorous shaking. EMS-mutagenized cells were washed once with 50 mM $NaHSO_3$.

Aliquots of the mutagenized cultures were spread on to artificial sea-water solidified with 1% agar. After 2 weeks of growth, colonies were individually inoculated into 2 ml artificial sea-water in tubes and incubated for 2 weeks. The cells were pelleted by centrifugation and the growth medium was removed. Fatty acid methyl esters (FAMEs) were prepared from whole cells with 0.5 ml C_6H_6 and 1 ml 3N methanolic HCl and analysed by GC as described [6].

Lipid extraction and analysis. Lipids were extracted as described previously [6]. PC and PI were sepd on high-performance silica gel TLC plates with $CHCl_3$–MeOH–H_2O (65:25:4). Other polar lipids were sepd in 2D by silica gel TLC using $CHCl_3$–MeOH–H_2O (65:25:4)

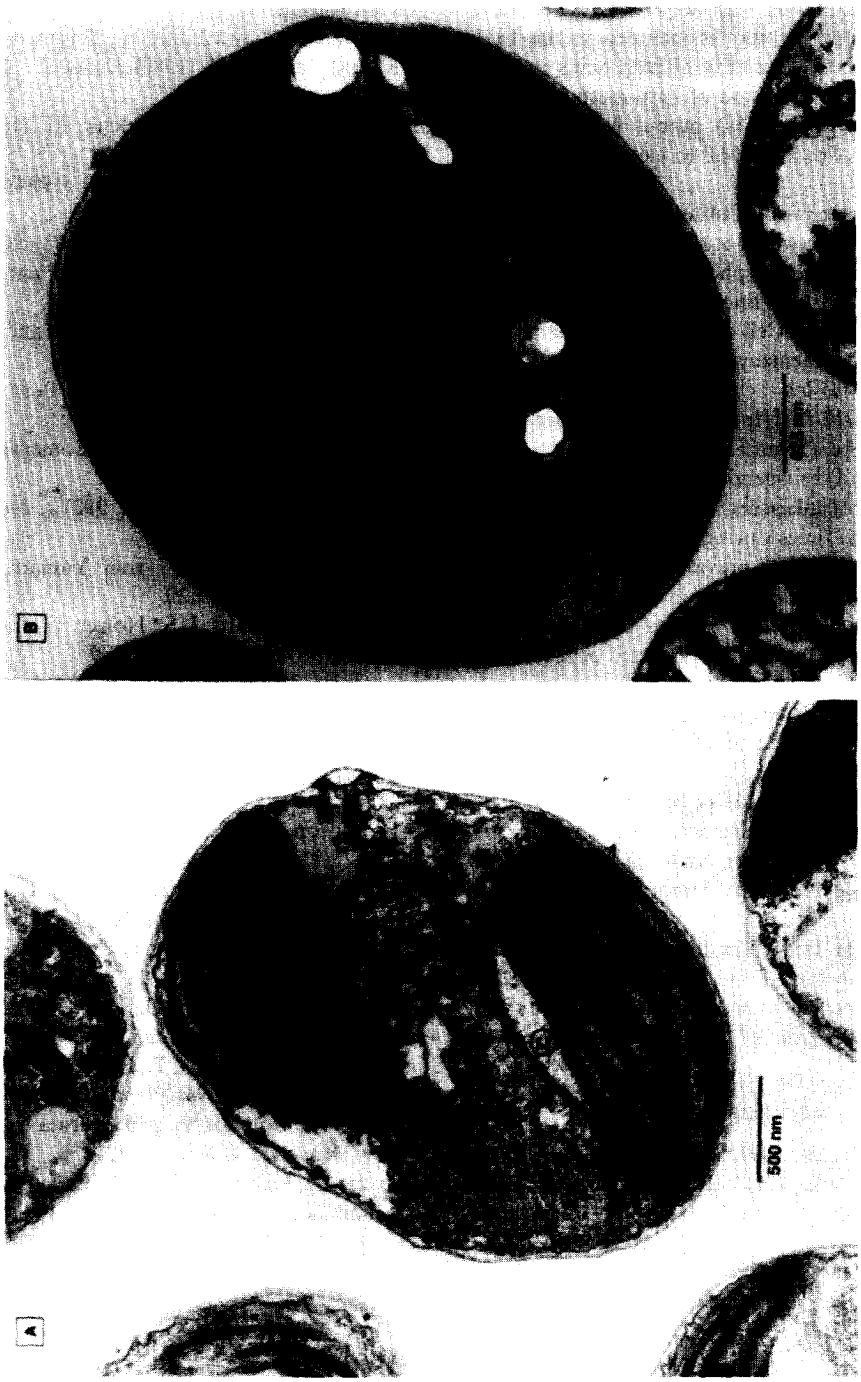

Fig. 6. Electron micrograph of the wild type (A) and mutant JS1 strain (B).

in the 1st dimension and $CHCl_3$–Me_2CO–HOAc–H_2O (25:10:5:5:2) in the 2nd. Lipids were located by light staining with I_2 vapour, then were scraped into tubes, transesterified, and analysed by GC as described above. [Small amounts of 16:1-*trans* were found in PG and SQDG in a previous study [6]. The GC column used in the present study did not separate the *cis* and *trans* isomers; thus, the *trans* isomer is included in the 16:1 (*n*-7) peak.] The relative quantity of fatty acids in each lipid was measured by inclusion of 19:0-methyl ester as an internal standard. The FID output from GC was adjusted by the M_r of each FAME to yield mole percentage.

Growth rate measurement. Cultures were initiated by inoculating the medium with 2×10^6 cells. Cultures were grown at 23° with 50 μmol photons $m^{-2} s^{-1}$ constant illumination from cool-light fluorescent bulbs and sparging with 1% CO_2 in air. Cell density was measured by counting cells with a haemacytometer.

Radiolabelling. Mid-log cultures (*ca* 6×10^7 cells ml^{-1}) were concd 10-fold to 20 ml and labelled with 20 μCi [^{14}C]NaOAc (54 mCi $mmol^{-1}$) for 1 hr. Cells were centrifuged and resuspended in unlabelled medium containing 0.25 mM unlabelled NaOAc. Aliquots were harvested at various times during the chase period, and lipids extracted as described previously [6].

MGDG molecular species analysis. MGDG was isolated and sepd into molecular species by HPLC with FID, and positional analysis of the collected peaks was as described in ref. [15].

Electron microscopy. Cells were prepd by a chemical fixation process as described in ref. [10].

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